SUPPLEMENTARY INFORMATION

Fig.S1. Click-IT assay of the palmitoylation level of PD-1.

A

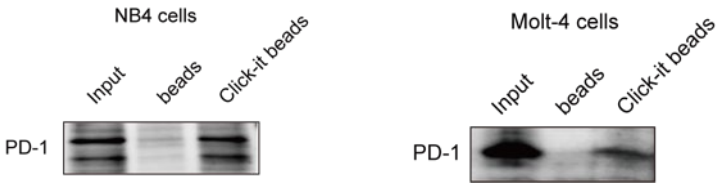

Fig.S1A. The Click-IT assay in NB4 (left panel) and Molt-4 cells (**right pane**) showing the same endogenous PD-1 band size in Click-IT beads compared to input lysate.

B

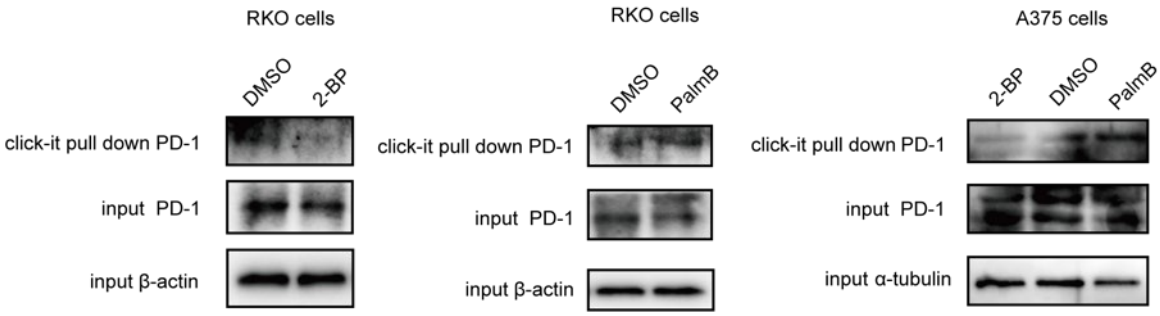

Fig.S1B. Click-IT assay of the palmitoylation level of PD-1 in RKO cells treated with 2-BP (left panel) or palmostatinB (middle panel) and in A375 cells treated with 2-BP or palmostatinB (right panel).

Fig.S2. Statistics of expression of PD-1 in a panel of cancer cell lines.

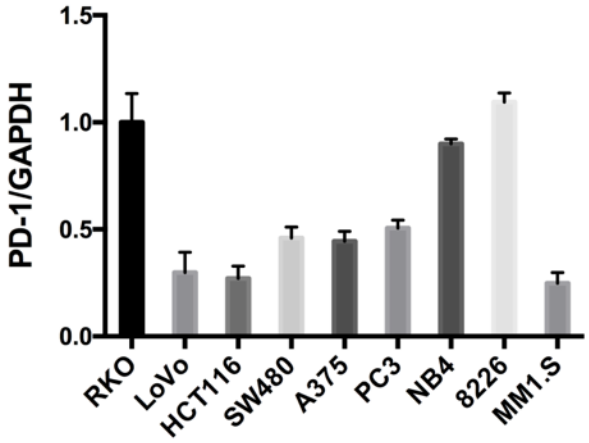

Fig.S2. Statistics of expression of PD-1 in a panel of cancer cell lines by western-blot using anti-PD-1 specific antibody from three independent experiments. n = 3 independent experiments.

Fig.S3 The mRNA expression of different DHHCs in the indicated cancer types, Western Blot results and quantification.

A

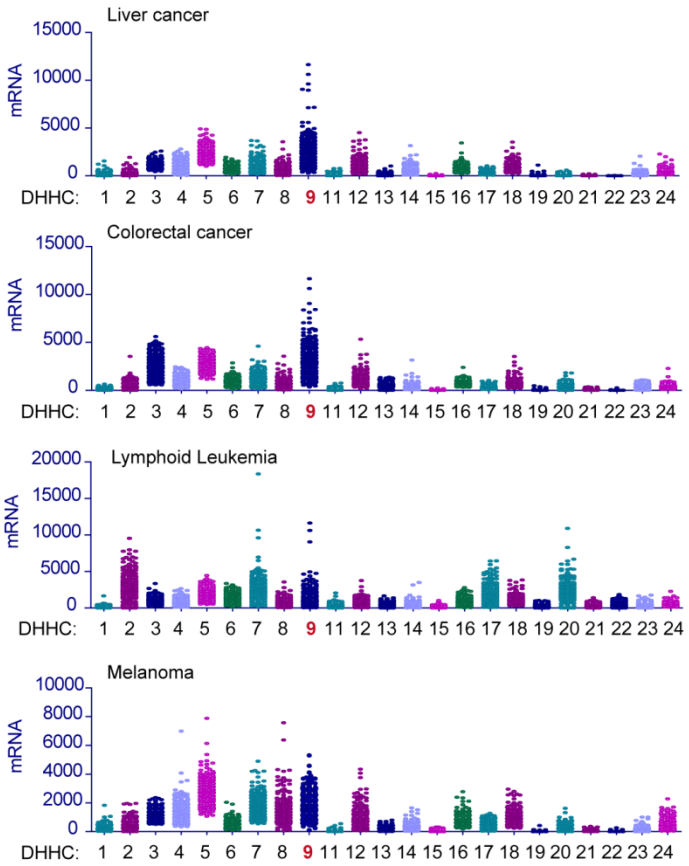

Fig.S3A. The mRNA expression of different DHHCs in the indicated cancer types, according to the mRNA expression data set of the CCLE (Cancer Cell Line Encyclopedia) project. The x-axis indicates the identity of DHHC (e.g., '9' indicates DHHC9), and the y-axis shows the mRNA expression level determined by RNA-seq.

B

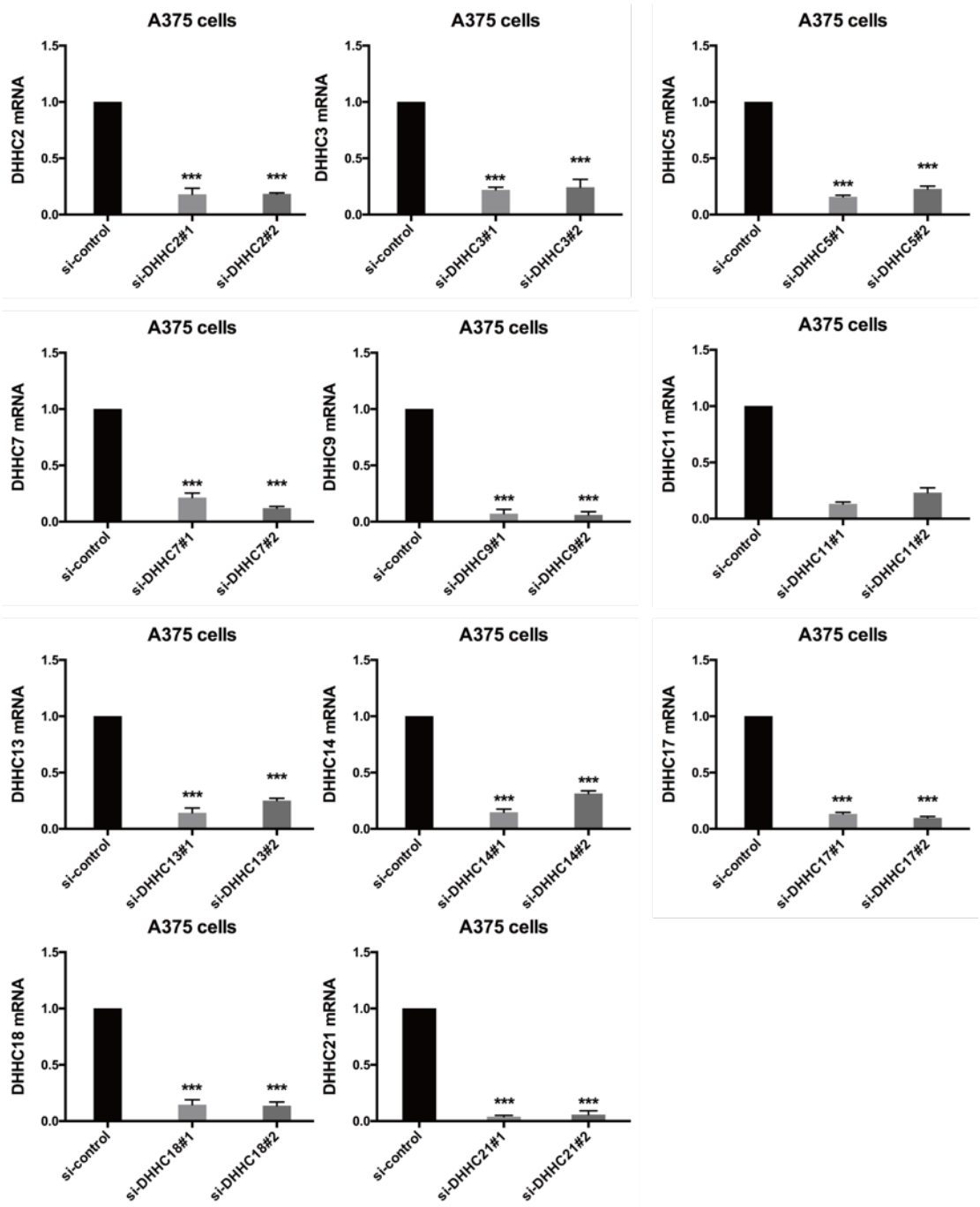

Fig.S3B. Real time PCR assay showing the mRNA expression of different DHHCs in A375 cells treated by two independent siRNAs targeting the indicated DHHC enzymes. *** P<0.001, ANOVA test. *n* = 3 independent experiments.

C

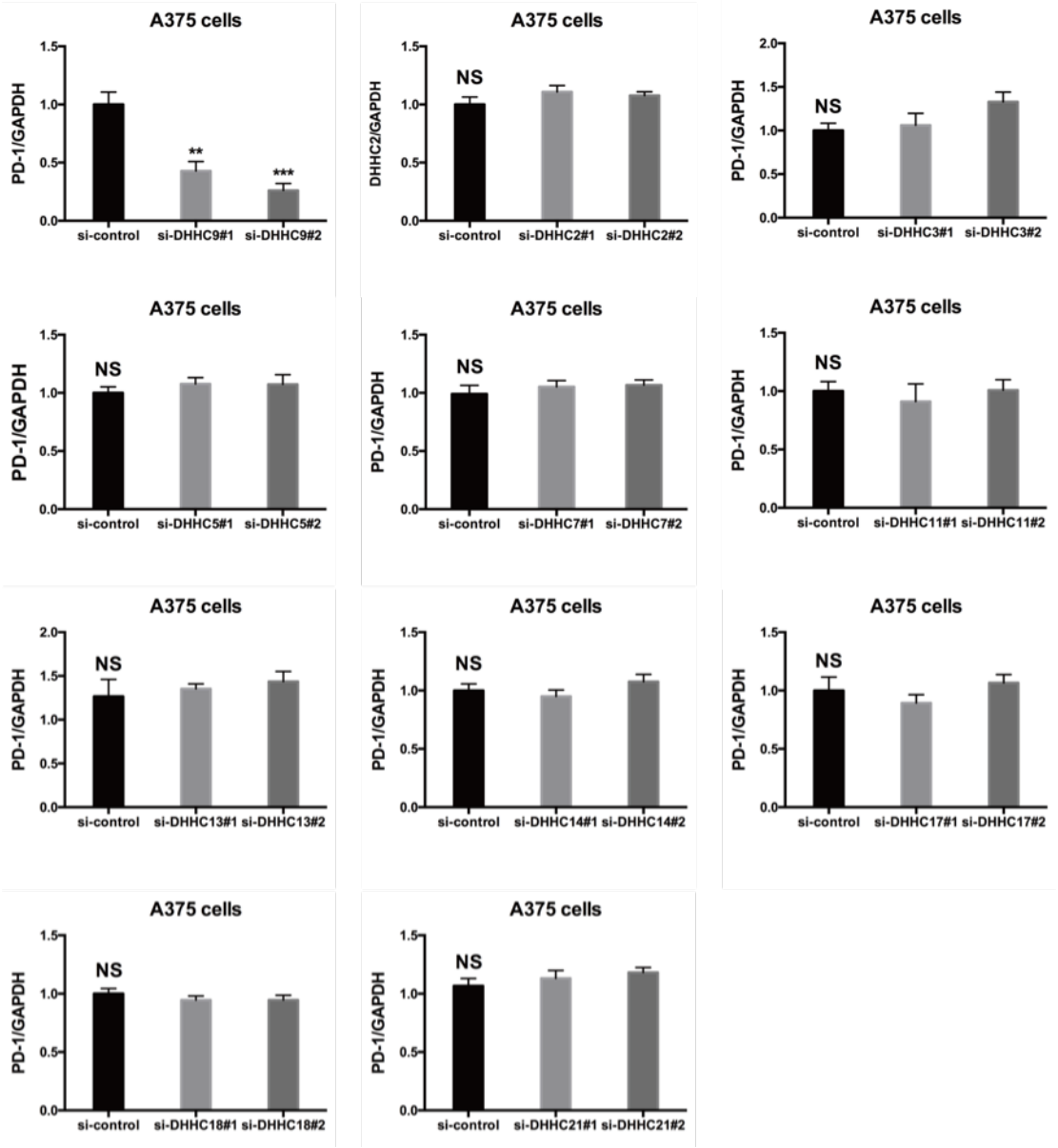

Fig.S3C. Statistics of western blot assay for DHHC screening in A375 cells from three independent experiments. *** P<0.001, ** P<0.01, NS, P>0.05, ANOVA test. *n* = 3 independent experiments.

D

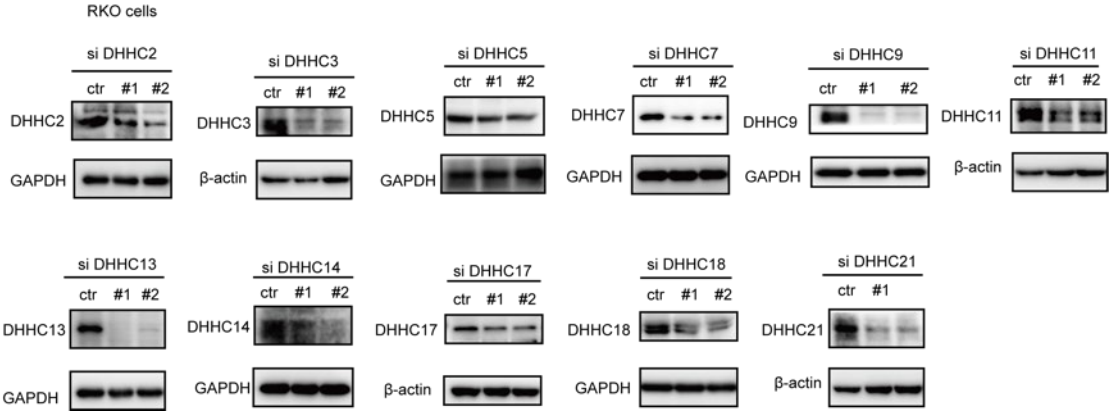

Fig.S3D. Western blot showing effect of two independent siRNAs targeting the indicated DHHC enzymes on DHHC protein levels in RKO cells with the specific antibody for each DHHC enzyme.

E

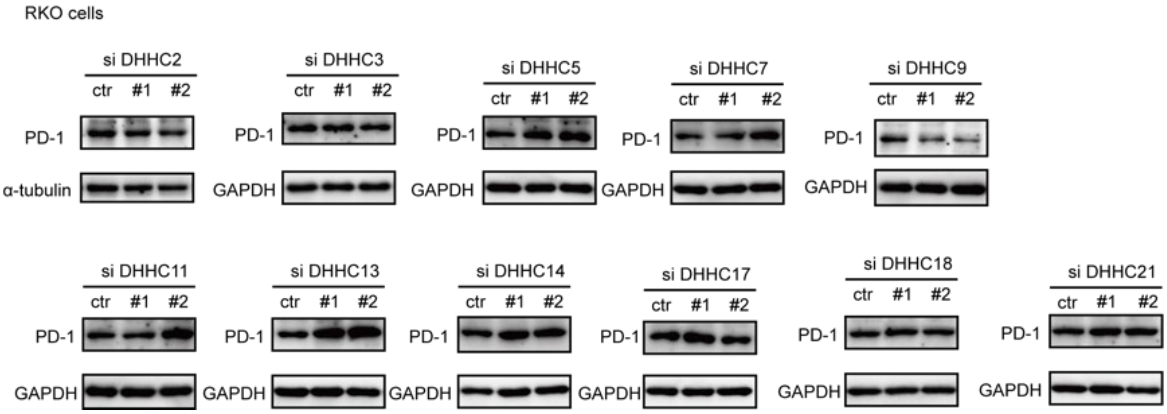

Fig.S3E. Western blot showing the expression of PD-1 in RKO cells treated by two independent siRNAs targeting the indicated DHHC enzymes.

F

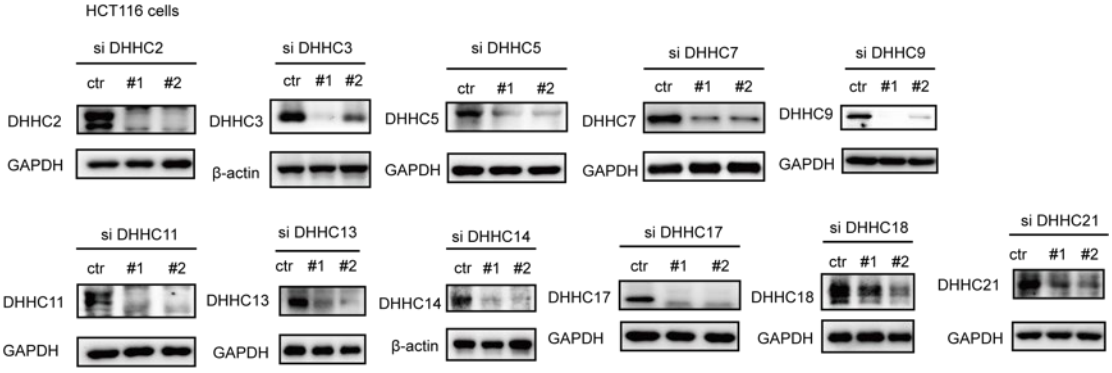

Fig.S3F. Western blot showing effect of two independent siRNAs targeting the indicated DHHC enzymes on DHHC protein levels in HCT116 cells with the specific antibody for each DHHC enzyme.

G

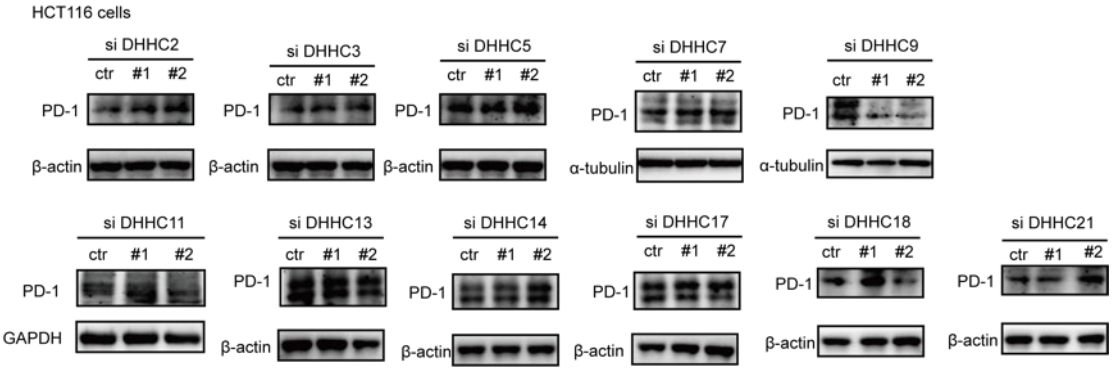

Fig.S3G. Western blot showing the expression of PD-1 in HCT116 cells treated by two independent siRNAs targeting the indicated DHHC enzymes.

H

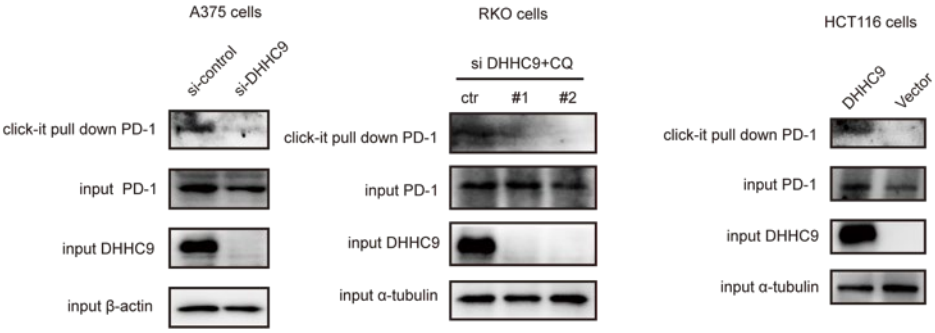

Fig.S3H. Click-IT assay of palmitoylation level of PD-1 in A375 cells (left panel) and RKO cells (middle panel) transfected with DHHC9 siRNA and in HCT116 cells transfected with DHHC9 plasmid.

I

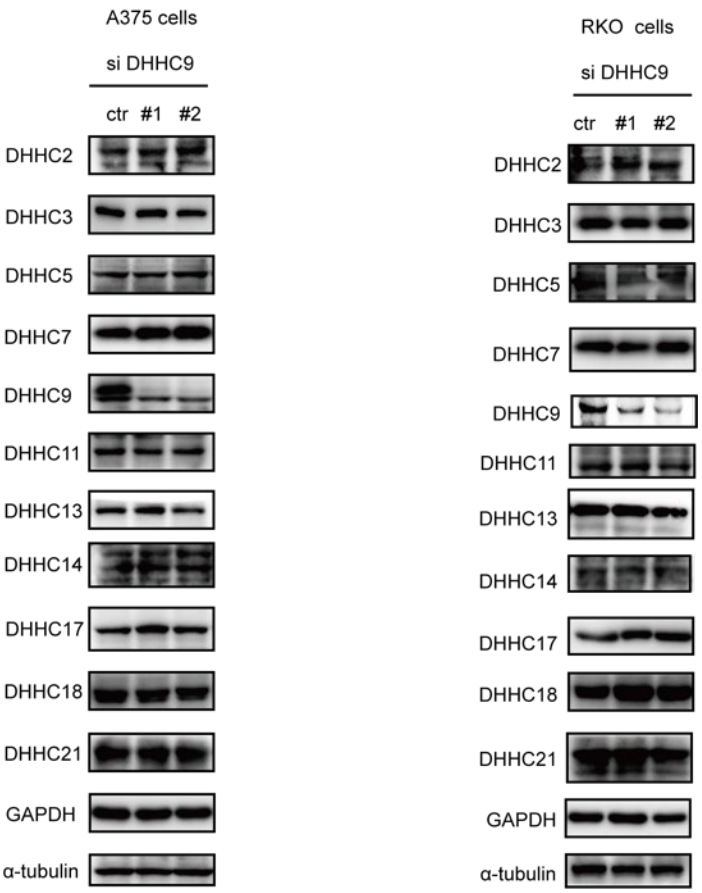

Fig.S3I. Western blot showing the expression of different DHHC enzymes with transfection of DHHC9 siRNA in A375 cells (left panel) and RKO cells(right panel).

Fig.S4. The degradation of PD-1 evaluated by cycloheximide (CHX)-chase assay

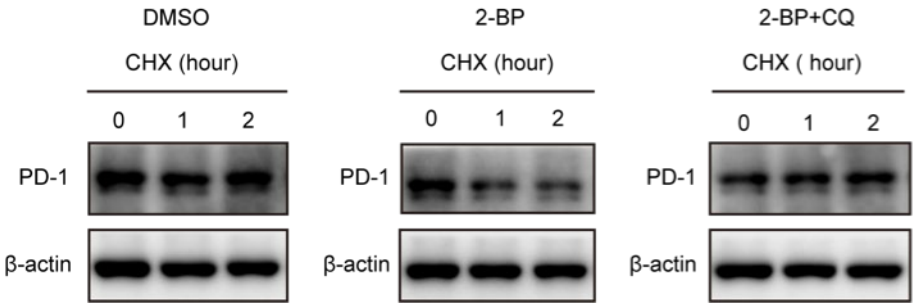

Fig.S4. The degradation of PD-1 was evaluated by cycloheximide (CHX)-chase assay. Treatment with 2-BP reduces PD-1 level and this decrease is rescued by the lysosomal inhibitor chloroquine (CQ).

Fig.S5. Quantification of western blotting assay from three independent experiments

A

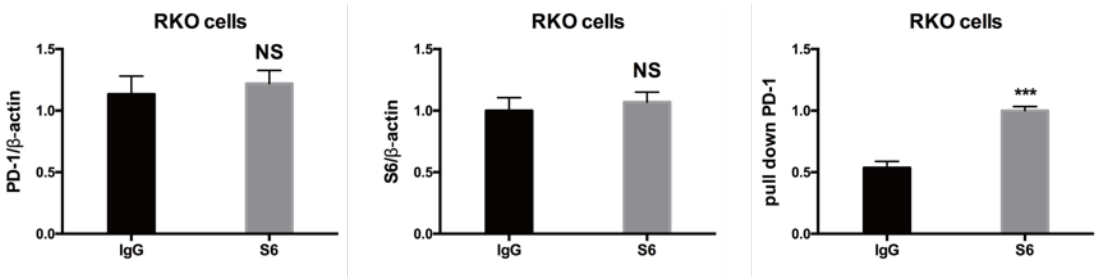

Fig.S5A. Quantification of western blotting assay from three independent experiments in Figure 5A.

B

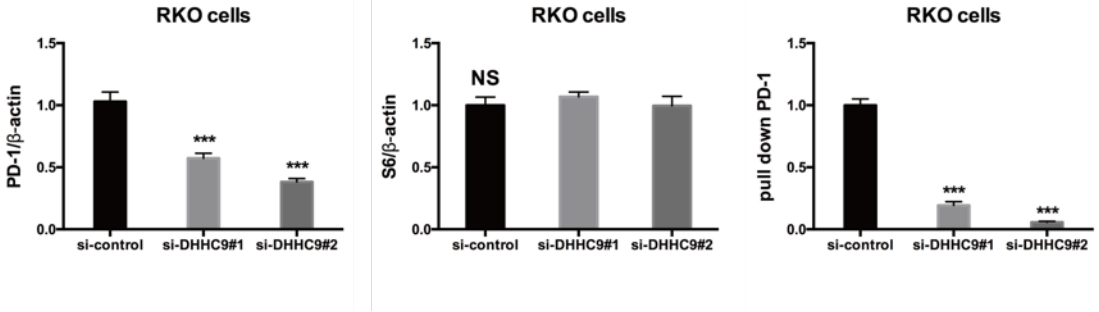

Fig.S5B. Quantification of western blotting assay from three independent experiments in Figure 5B. *** P<0.001, NS, P>0.05, ANOVA test. *n* = 3 independent experiments.

C

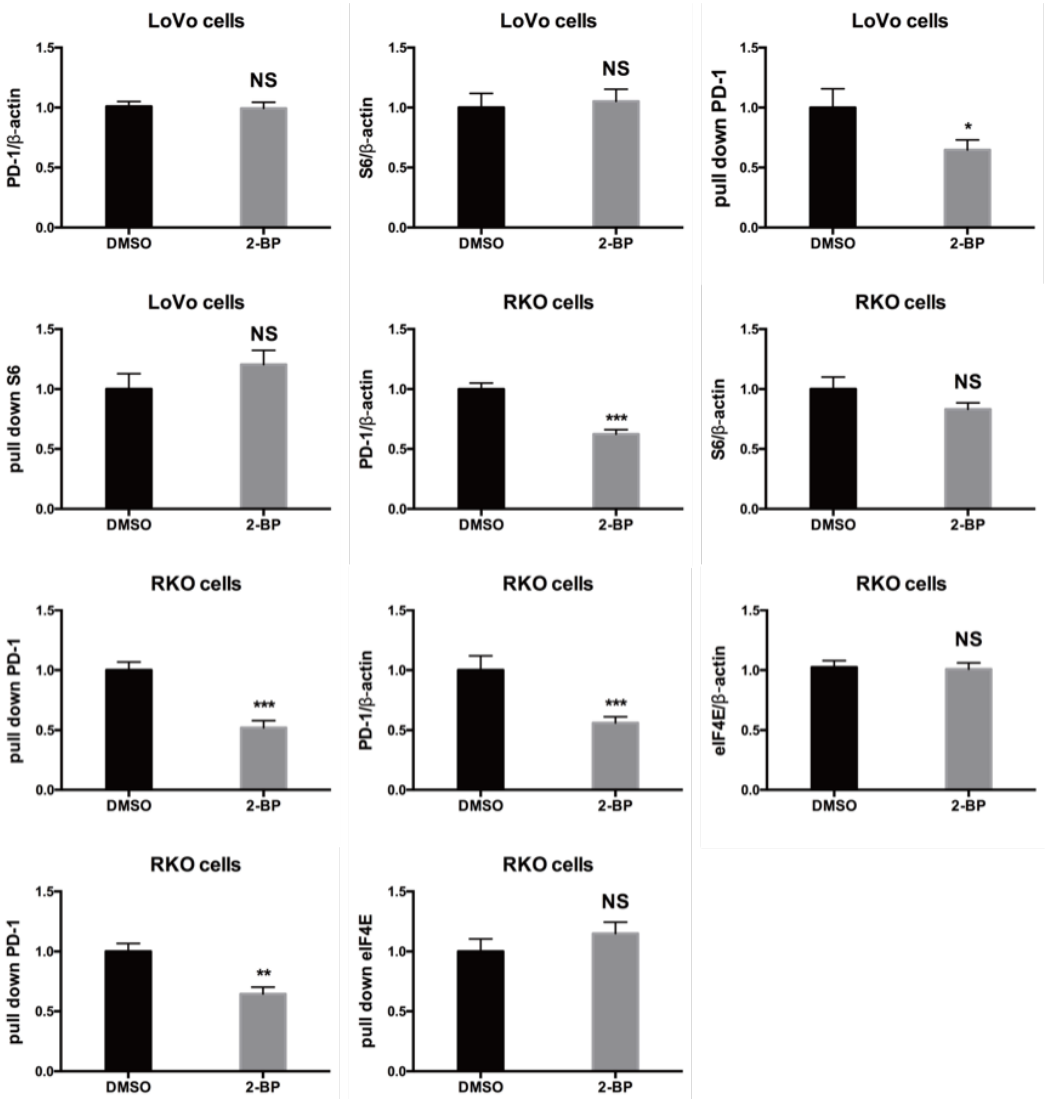

Fig.S5C. Quantification of western blotting assay from three independent experiments in Figure 5C. *** P<0.001, ** P<0.01, * P<0.05, NS, P>0.05, ANOVA test. *n* = 3 independent experiments.

D

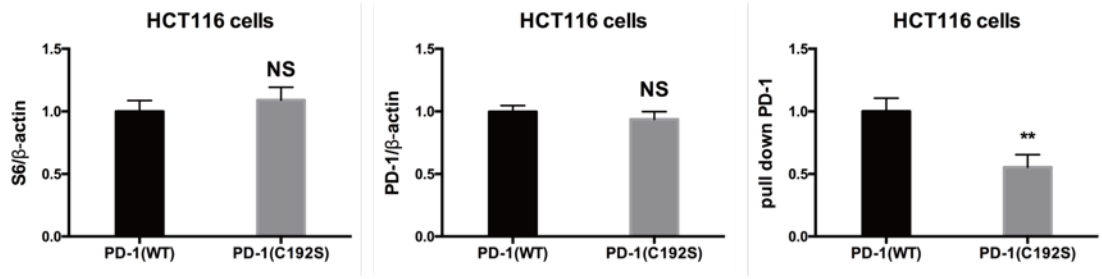

Fig.S5D. Quantification of western blotting assay from three independent experiments in Figure 5D. ** P<0.01, NS, P>0.05, ANOVA test. *n* = 3 independent experiments.

E

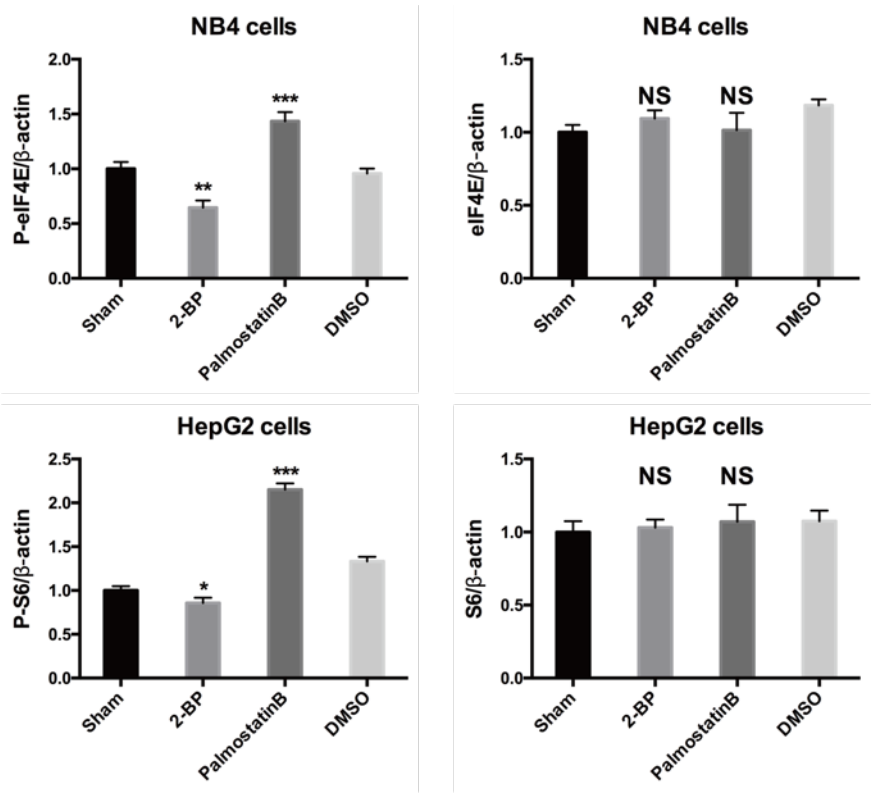

Fig.S5E. Quantification of western blotting assay from three independent experiments in Figure 5E. *** P<0.001, ** P<0.01, * P<0.05, NS, P>0.05, ANOVA test. *n* = 3 independent experiments.

F

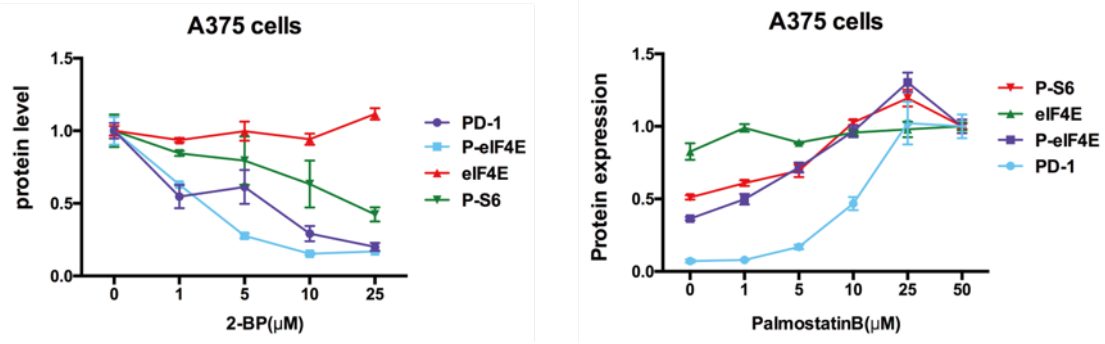

Fig.S5F. Quantification of western blotting assay from three independent experiments in Figure 5F.

G

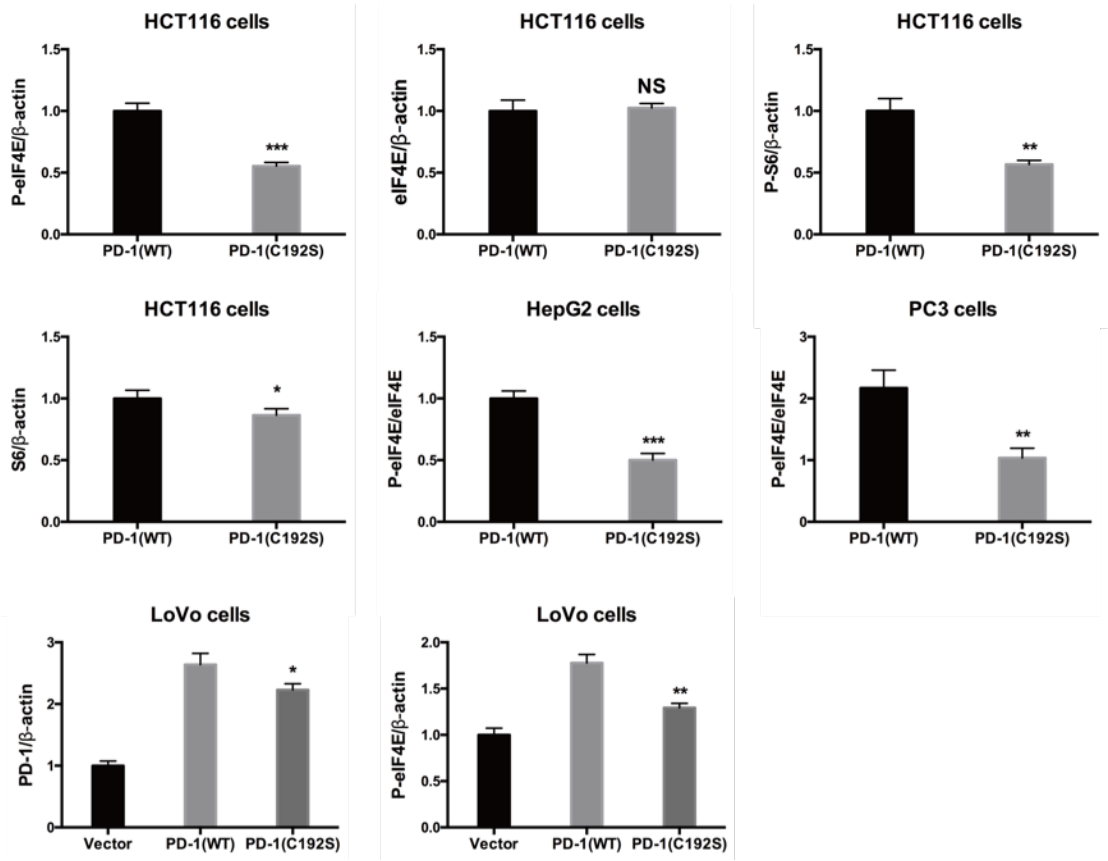

Fig.S5G. Quantification of western blotting assay from three independent experiments in Figure 5G. *** P<0.001, ** P<0.01, * P<0.05, NS, P>0.05, ANOVA test. *n* = 3 independent experiments.

Fig.S6. Anchorage-free colony formation and Western Blot assays.

A

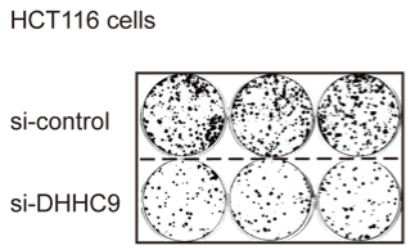

Fig.S6A. Silencing DHHC9 decreases anchorage-free colony formation compared to control conditions.

B

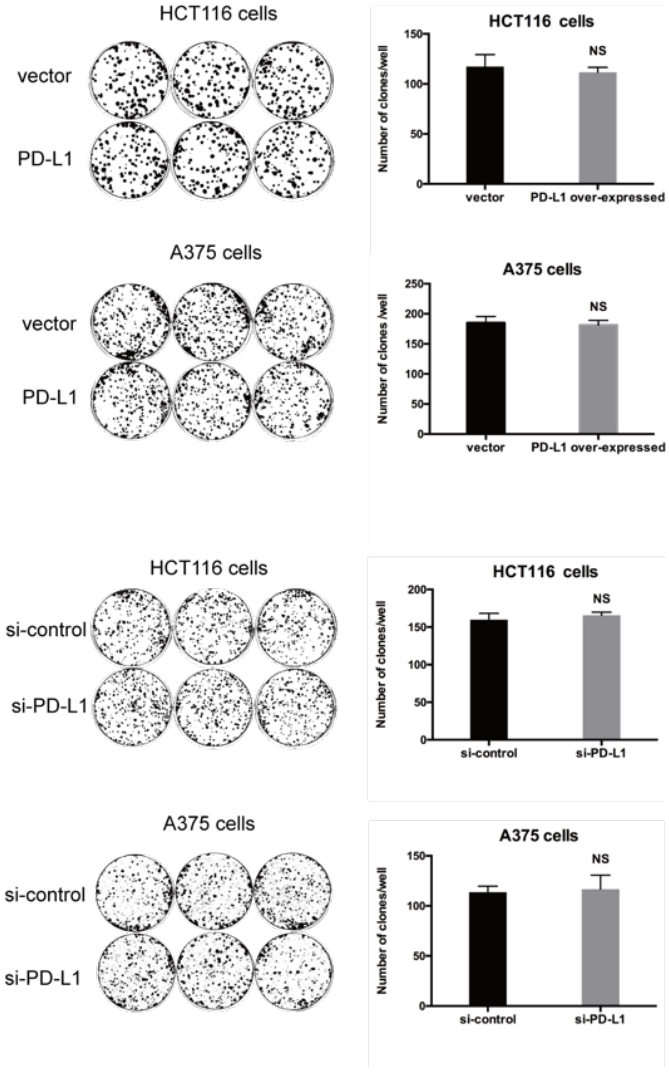

Fig.S6B. Clone formation assay showing that overexpressing or silencing PD-L1 does not affect tumor cell growth. NS, P>0.05, ANOVA test. *n* = 3 independent experiments.

C

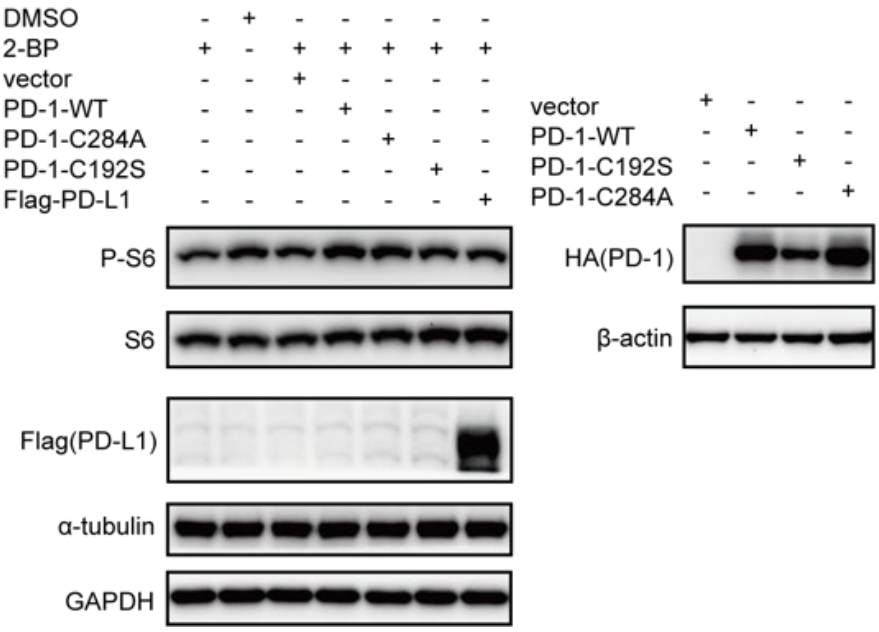

Fig.S6C. Western blot assay showing that PD-1 and its C284A mutant but not its C192S mutant or PD-L1 can rescue the effect of 2-BP on reducing phosphorylation level of S6.

Fig.S7. Western bolt assay showing the effect of PD1-PALM.

A

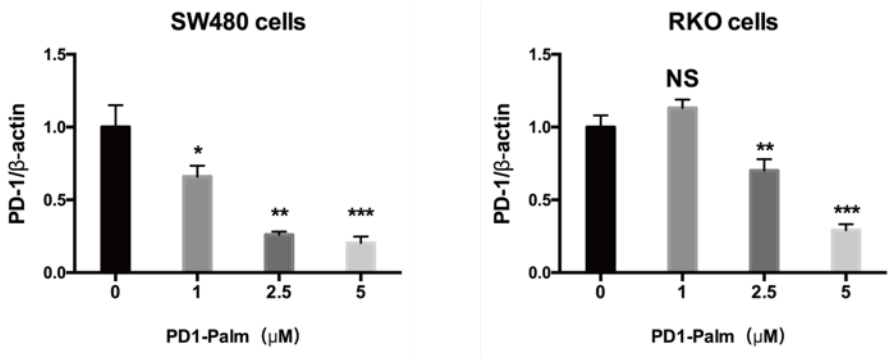

Fig.S7A. Quantification of the western blotting assay in Figure 7C from three independent experiments. *** P<0.001, ** P<0.01, * P<0.05, NS, P>0.05, ANOVA test. *n* = 3 independent experiments.

B

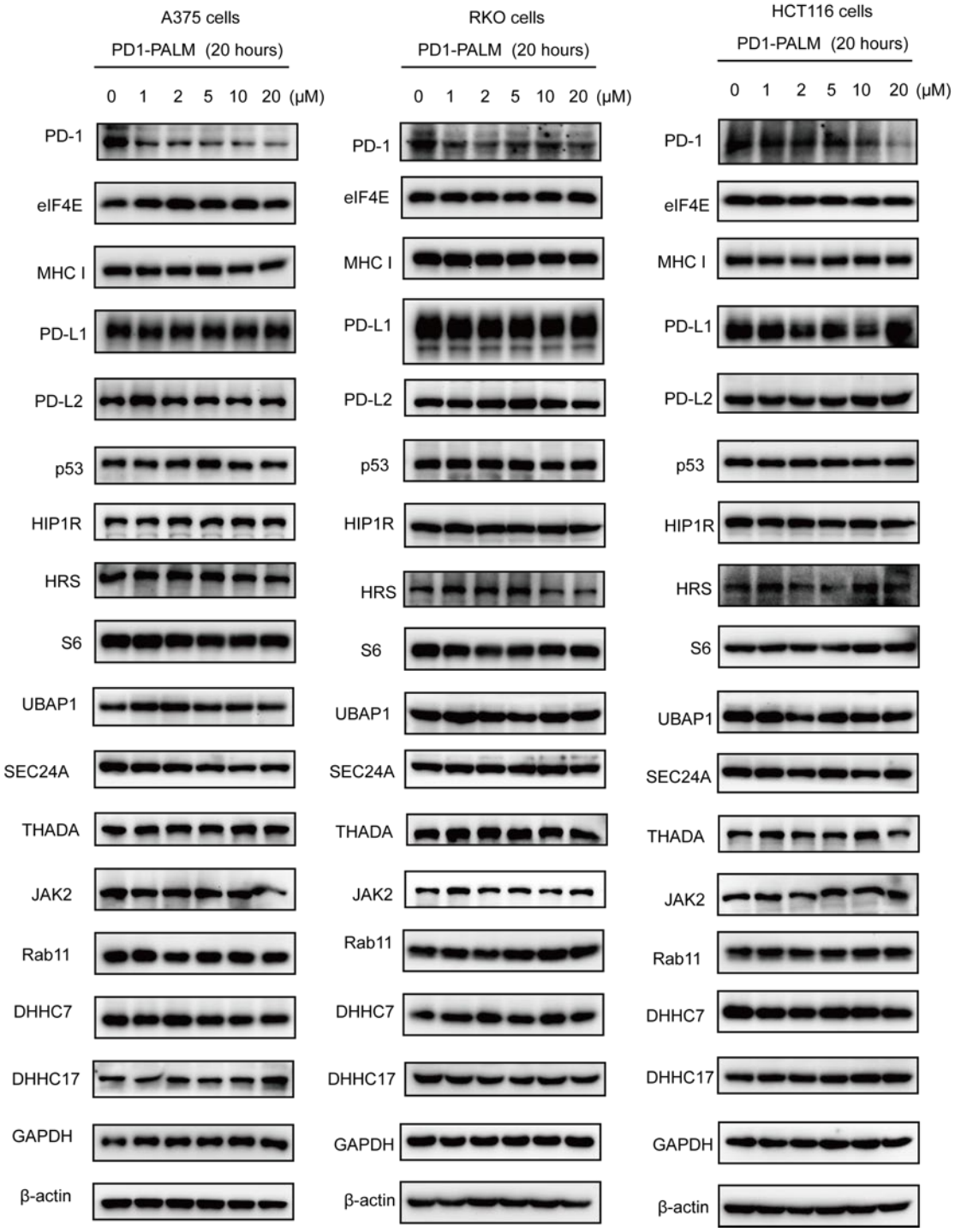

Fig.S7B. Western bolt assay showing the effect of PD1-PALM on the expression of other seventeen proteins besides PD-1.

Supplementary Tables.

Table S1. Sequences of siRNAs used in this study.

Name	Sense	Antisense
si-DHHC3#1	CCACAGUGAUUCUCCUUAUTT	AUAAGGAGAAUCACUGUGGTT
si-DHHC3#2	CCUCAAAGUGGAUUCACUUTT	AAGUGAAUCCACUUUGAGGTT
si-DHHC5#1	CCAGACUCAGUGGACAUUUTT	AAAUGUCCACUGAGUCUGGTT
si-DHHC5#2	GGCUCAUUGGCAUUUAGUUTT	AACUAAAUGCCAAUGAGCCTT
si-DHHC5#3	GCUCAUUGGCAUUUAGUUUTT	AAACUAAAUGCCAAUGAGCTT
si-DHHC2#1	AAGGAUCUUCCCAUCUAUUTT	AAUAGAUGGGAAGAUCCUUGG
si-DHHC2#2	GGAUCUUCCCAUCUAUACCTT	GGUAUAGAUGGGAAGAUCCTT
si-DHHC7#1	CCGGGACGUCGAGCAUCAUTT	AUGAUGCUCGACGUCCCGGTT
si-DHHC7#2	GCAGACUUCGUGGUGACUUTT	AAGUCACCACGAAGUCUGCTT
si-DHHC7#3	GCGCCCACCACUGCAGUAUTT	AUACUGCAGUGGUGGGCGCTT.
si-DHHC9#1	GGGACUGACUGGAUUUCAUTT	AUGAAAUCCAGUCAGUCCCTT
si-DHHC9#2	GGAAGAAUCGCGUCCAGAATT	UUCUGGACGCGAUUCUUCCTT
si-DHHC9#3	CCAGAAUCCCUACAGCCAUTT	AUGGUCGUAGGGAUUCUGGTT
si-DHHC11#1	GCAGGUGCAGACCCUGAUATT	UAUCAGGGUCUGCACCUGCTT
si-DHHC11#2	GCAGUGAGGAAAGAUCCAUTT	AUGGAUCUUUCCUCACUGCTT
si-DHHC11#3	GCAGGCGCCUGUGUCAGUUTT	AACUGACACAGGCGCCUGCTT
siDHHC14#1	CAAGCCUGAUCGACAGAAGAGGGUA	UACCCUCUUCUGUCGAUCAGGCUUG
siDHHC14#2	GACCAGUGCAUUCAGAGCACCAAAU	AUUUGGUGCUCUGAAUGCACUGGUC
si-DHHC13#1	GCAUCCACCUGGCAGUAUUTT	AAUACUGCCAGGUGGAUGCTT
si-DHHC13#2	GGUUGGGUAUAAGAACCUUTT	AAGGUUCUUAUACCCAACCTT
si-DHHC13#3	GGACAUCACAGUACACCAUTT	AUGGUGUACUGUGAUGUCCTT
si-DHHC17#1	GGAUGUAGAUAUGAUGGAUTT	AUCCAUCAUAUCUACAUCCTT;
si-DHHC17#2	GCAGCAUAUAGAACACAUATT	UAUGUGUUCUAUAUGCUGCTT
si-DHHC17#3	GCUACAGUACAGUUUCUUUTT	AAAGAAACUGUACUGUAGCTT
si-DHHC18#1	CCGGCCUCUUCUUCGUCUUT	AAGACGAAGAAGAGGCCGGTT
si-DHHC18#2	GCGCUCAGGGAAGCAACUUTT	AAGUUGCUUCCCUGAGCGCTT
si-DHHC18#3	CCAAGCCUGAUGCCAGCAUTT	AUGCUGGCAUCAGGCUUGGTT
si-DHHC21#1	GCGUAAUUUGGACCUCUUUTT	AAAGAGGUCCAAAUUACGCTT
si-DHHC21#2	GCAGCCUUUAUGGGCAUUATT	UAAUGCCCAUAAAGGCUGCTT
si-DHHC21#3	CCAAUCUUAGGCAUCAUAATT	UUAUGAUGCCUAAGAUUGGTT.
si-Negative control	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT